Safety, Reactogenicity, and Immunogenicity of Human Rotavirus Vaccine RIX4414 in Human Immunodeficiency Virus-positive Infants in South Africa

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Background: Rotavirus and human immunodeficiency virus (HIV) infections are a cause of great public health concern in developing countries. The current study evaluated the safety, reactogenicity, and immunogenicity of RIX4414 vaccine in asymptomatic or mildly symptomatic (clinical stages I and II according to WHO classification) HIV-infected South African infants.

Methods: A total of 100 HIV-positive infants aged 6 to 10 weeks enrolled in this double-blind, 1:1 randomized, placebo-controlled study were allocated into 2 groups to receive 3 doses of RIX4414 vaccine/placebo according to a 0-, 1-, and 2-month schedule. Routine vaccines were concomitantly administered. Solicited and unsolicited symptoms were recorded for 15 and 31 days after each dose, respectively. Serious adverse events were recorded throughout the study period. Serum antirotavirus IgA concentrations (enzyme-linked immunosorbent assay, cut-off ≥20 U/mL) and the immunodeficiency status were determined at screening and 2 months post-Dose 3. Stool samples were analyzed for rotavirus using enzyme-linked immunosorbent assay at predetermined points and during diarrhea episodes.

Results: All symptoms (solicited and unsolicited) occurred at a similar frequency in both groups. Six fatal serious adverse events in RIX4414 and 9 in placebo groups were reported. At 2 months post-Dose 3, the seroconversion rates were 57.1% (95% CI: 34–78.2) in RIX4414 and 18.2% (95% CI: 5.2–40.3) in the placebo group. The mean absolute $CD4^+$ cell count, $CD4^+$ percentage, and HIV-1 viral load were comparable in both groups at screening and 2 months post-Dose 3. Rotavirus shedding peaked at Day 7 after Dose 1 of RIX4414 with prolonged shedding was observed in 1 infant only.

Conclusions: Three doses of RIX4414 vaccine was tolerated well by the South African HIV-positive infants. A satisfactory immune response was mounted without aggravating their immunologic or HIV condition.

Key Words: RIX4414, rotavirus vaccine, HIV-positive infants, South Africa

(*Pediatr Infect Dis J* 2011;30: 125–130)

In the developing world, diarrhea is one of the most important causes of deaths and hospitalizations in children younger than 5 years; more than 1 billion diarrhea episodes and almost 2 million deaths are reported in this age group every year.[1,2] According to estimates of the World Health Organization (WHO), in Africa alone, diarrhea accounts for 16% of deaths in children aged less than 5 years.[1,3]

Of all the enteric pathogens responsible for acute diarrhea in developing countries worldwide, rotavirus has been established as the leading cause of severe dehydrating diarrhea with an estimated 527,000 deaths per year in children aged <5 years across the globe.[4,5] This global trend of rotavirus being the main causal agent for diarrhea-related deaths and hospitalization has also been observed in Africa, with rotavirus accounting for one-third of all hospital admissions in African infants.[6] On the basis of recent efficacy data generated with RIX4414 in African countries, the WHO Strategic Advisory Group of Experts has recommended the global introduction of rotavirus vaccines into the routine childhood immunization program.[7]

In addition to diarrheal diseases, human immunodeficiency virus (HIV) infection is a major cause of public health concern in developing countries across the globe, especially where HIV infection is highly endemic. High HIV prevalence coupled with substantial rotavirus disease burden is a critical public-health problem in sub-Saharan Africa as both these infections contribute to high childhood mortality rates. Also, HIV-positive infants could represent a substantial percentage of the population in this region that would require immunization against rotavirus infections. In addition, some of these infants may experience extreme comorbidity due to the HIV infection and develop severe, chronic, and sometimes fatal rotavirus infections.[8,9] Therefore, WHO requested the evaluation of the live, attenuated rotavirus vaccines in this HIV-infected infant population, to establish the safety of the live rotavirus vaccine.[10]

To mitigate the global rotavirus disease burden, an oral live-attenuated human rotavirus vaccine RIX4414 was developed and its safety and efficacy is established in healthy infants across various continents, including Africa.[11–14] On the basis of clinical data from an earlier study conducted in South Africa where HIV-positive infants were enrolled jointly with other HIV-negative infants, an independent data monitoring committee and the

Accepted for publication July 27, 2010.

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Supported by research grants from the World Health Organization (V27/181/173), the Program for Appropriate Technology in Health (PATH Grant GAV.1142–01–07211-SPS), the Norwegian Program for Development, Research and Higher Education research grant (PRO 48/2002), and the South African Medical Research Council. GlaxoSmithKline Biologicals was also the funding source and was involved in all stages of the study conduct and analysis. GSK Biologicals also took in charge all costs associated with the development and the publishing of the present manuscript.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/11/3002-0125
DOI: 10.1097/INF.0b013e3181f42db9

WHO recommended further evaluation of safety of RIX4414 vaccine in the HIV-infected infant population.[6,10,15]

Currently, 2 oral doses of RIX4414 vaccine are administered at 2 and 4 months of age; however, at the time of this study, the number of doses to be administered to infants from low socioeconomic background and of very low age was still under evaluation.[16,17] In addition, if safety was to be evaluated in a developing country setting, the assessment of the longest possible regimen (ie, 3-dose schedule) was critical to consider. Therefore, the present study was conducted in asymptomatic or mildly symptomatic (clinical stages I and II according to WHO classification)[18] HIV-infected infants from South Africa to evaluate the safety, reactogenicity, and immunogenicity of 3 doses of RIX4414 vaccine as compared with placebo, when administered concomitantly with routine vaccines including oral polio vaccine (OPV).

MATERIALS AND METHODS

Study Design

This randomized (1:1) double-blinded study (Study ID: NCT00263666) was conducted in South African infants in accordance with Good Clinical Practice guidelines and the 1996 version of Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committees of the institutions (University of Limpopo and University of the Witwatersrand) and the WHO. Written consent was obtained from the parents/guardians of infants before any study procedures were performed.

The HIV status of infants was assessed at the screening visit (5–9 weeks of age) (assessed by qualitative HIV DNA PCR [AMPLICOR HIV-Qualitative Test, version 1.5; Roche Diagnostics, NJ]) prior to study entry. After the confirmation of HIV infection in infants, the immune categorization in terms of $CD4^+$ cell count was performed. Log10 HIV-1 viral load and serum antirotavirus IgA antibody concentrations were assessed at the time of screening visit. Only HIV-positive infants (confirmed at screening) who were clinically asymptomatic or mildly symptomatic (clinical stages I and II according to WHO classification)[18] and aged 6 to 10 weeks at the time of Dose 1 of RIX4414/placebo were enrolled and randomly allocated into 2 groups (RIX4414 group and placebo group) to receive 3 oral doses of RIX4414 vaccine/placebo according to a 0-, 1-, 2-month schedule. RIX4414 vaccine/placebo was concomitantly administered with 3 doses of combined diphtheria, tetanus and whole-cell pertussis, hepatitis B, and *Haemophilus influenzae* type b vaccine (Tritanrix−HepB+Hib) and OPV (PolioSabin). As this study was conducted in HIV-positive infants, the independent data monitoring committee regularly reviewed all reported serious adverse events (SAEs). Infants were not included in the study if they were confirmed HIV-negative, had received any other investigational drug or vaccine 30 days before receiving the first dose of study vaccine, or had a history of chronic gastroenteritis (GE) or previous documented rotavirus GE.

For infants who developed clinical symptoms of HIV (WHO stages III or IV disease) anytime after enrolment, access to antiretroviral therapy (cotrimoxazole) according to the South African national guidelines was facilitated. Infants who needed treatment were referred to antiretroviral therapy centers by the investigators.

Vaccine

The lyophilized formulation of RIX4414 (Rotarix), buffer and placebo were developed and manufactured by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. Each dose of the vaccine contained at least $10^{6.0}$ median cell culture infective dose ($CCID_{50}$) of the active virus strain. The placebo was similar to RIX4414 in appearance and contained the same constituents as the active vaccine except that it did not contain the vaccine virus.

Assessment of Safety and Reactogenicity

All solicited general symptoms (fever, fussiness/irritability, diarrhea, vomiting, loss of appetite, cough/runny nose) and unsolicited symptoms were recorded during the 15-day and 31-day postvaccination follow-up period after each RIX4414/placebo dose, respectively. The intensity of adverse events was assessed on a 4-point scale, where "0" indicated no symptoms; "1," mild; "2," moderate; and "3" severe symptoms. Symptoms of Grade 3 intensity were defined as follows: rectal temperature of >39.5°C (fever), ≥6 looser than normal stools per day (diarrhea), ≥3 episodes of vomiting per day (vomiting), refusing food intake (loss of appetite), and preventing normal activity (cough/runny nose, fussiness/irritability). Grade 2 symptoms were defined as rectal temperature of >38.5°C to ≤39.5°C (fever), 4 to 5 looser than normal stools/d (diarrhea), 2 episodes of vomiting/d (vomiting), eating lesser than usual, which interfered with normal activity (loss of appetite), and interfering with normal activity (cough/runny nose, fussiness/irritability). Occurrence of SAEs was recorded throughout the study period.

Assessment of GE Episodes

A GE episode was defined as diarrhea (3 or more, loose than normal stools per day) with or without vomiting. Stool samples were collected between Dose 1 and 2 months post-Dose 3 at predetermined time points. The study protocol was amended to incorporate the changes in the stool sampling time-points. Therefore, for infants enrolled before protocol amendment 2, stool samples were collected on Days 0, 7, 15, and 22 of Doses 1 and 2 and on Days 0, 7, 15, 30, 45, and 60 of Dose 3. Stool samples of infants enrolled after protocol amendment 2 (considered for analysis) were collected on Days 0, 7, 14, and 21 of Doses 1 and 2 and on Days 0, 7, 14, 28, and 42 of Dose 3. If a subject shed rotavirus on Day 42 post-Dose 3, subsequent stool samples were collected at 2 months post-Dose 3 and every 2 weeks after that until rotavirus was no longer detected in stool samples by enzyme-linked immunosorbent assay(ELISA).

Stool samples were also collected from infants during each diarrhea episode that occurred throughout the study period. All stool samples (planned and diarrheal) were tested for rotavirus using ELISA method designed by Ward et al and adapted by GSK Biologicals[19,20] at the Medical Research Council unit, University of Limpopo (Medunsa), Pretoria, and at GSK designated laboratory (Dr. Richard Ward, Laboratory of Specialized Clinical Studies, Cincinnati Children's Hospital Medical Center, Cincinnati, OH). If the stool samples (planned or diarrheal) tested positive for rotavirus, they were further examined using reverse transcription polymerase chain reaction (RT-PCR) followed by gel detection to determine the G and P type at Medunsa.[21] Stool samples positive for G1 type at Medunsa were sent to DDL Diagnostic Laboratory to discriminate G1 wild-type and G1 vaccine type using RT-PCR and reverse hybridization. For untypable rotavirus types, DDL performed sequence analysis.[22]

Assessment on Immunogenicity

Blood samples were collected at screening and 2 months post-Dose 3 to measure antirotavirus IgA concentrations in RIX4414 and placebo groups (GSK Biologicals Laboratory, Belgium, using a well-established in-house ELISA [cut-off = 20 U/mL]). In this study, the postvaccination blood samples were collected approximately 2 months post-Dose 3 (as opposed to 1 month postlast Dose in other studies) to have a longer safety follow-up in this HIV-positive population.

Vaccine take, defined as serum antirotavirus IgA concentration ≥20 U/mL in postvaccination sera or rotavirus vaccine shedding in any stool sample collected from Dose 1 to 2 months post-Dose 3 for infants initially negative for rotavirus, was also recorded. The number of infants with rotavirus in stool samples (shedding) was collected and recorded from Dose 1 of RIX4414/placebo until rotavirus excretion ceased.

Assessment of Immune Deficiency Condition

All HIV assays were performed at Contract Laboratory Services, Johannesburg, South Africa. Blood samples were collected during the screening visit and 2 months post-Dose 3 to determine the HIV-1 viral load (COBAS AMPLICOR HIV-1 MONITOR Test, version 1.5; Roche Diagnostics, NJ), absolute $CD4^+$ cell count (automated full blood analyzer, Contract Laboratory Services, Johannesburg, South Africa) and $CD4^+$ percentage.[23] On the basis of absolute $CD4^+$ cell count and $CD4^+$ percentage, the HIV-positive infants were stratified into 3 immunologic categories as per Centers for Disease Control and prevention recommendations.[24]

Statistical Analyses

Statistical analyses were performed using SAS 9.1, and Proc StatXact-7 was used to calculate 95% confidence interval (CI).

A sample size of 100 infants (50 per group) was determined to demonstrate with 80% power, a 26% to 30% difference between the RIX4414 and placebo groups in the incidence of at least 1 Grade 2 or 3 fever, vomiting, or diarrhea during the 15-day follow-up period, considering an alpha of 5%. As this study was the first of its kind to be conducted on exclusively HIV-positive infants, a decision was taken to keep the sample size low but adequate to interpret results.

Safety analysis was done on the total vaccinated cohort for whom it was documented that at least 1 dose of study vaccine was administered. The incidence of all and Grade 3 solicited general symptoms, Grade 2 or 3 fever, vomiting, or diarrhea was calculated per infant with exact 95% CI. Unsolicited symptoms (all and Grade 3) were coded following the Medical Dictionary for Regulatory Activities and tabulated with 95% CI.

For the immunogenicity analysis, the according-to-protocol (ATP) cohort was considered. The ATP immunogenicity cohort comprised of infants who had complied with the protocol and for whom immunogenicity data at both points were available. Seroconversion rate (antirotavirus IgA antibody concentration ≥20 U/mL), vaccine take, and geometric mean concentration (GMC) were calculated with 95% CI.

For analysis of the immunodeficiency condition, the percentage of infants belonging to each immunologic category was tabulated with 95% CI in both groups. Furthermore, the median absolute $CD4^+$ cell counts, median $CD4^+$ percentage, and median log10 HIV-1 viral load were also tabulated.

RESULTS

Demography

This study was conducted on 100 HIV-positive infants (50 each in RIX4414 and placebo groups) between March 2005 and February 2008. Of these, 2 infants had tested positive for HIV initially with HIV DNA PCR testing, but tested negative in the subsequent confirmatory log10 HIV-1 viral load test. These HIV-negative infants remained in the study but were eliminated from the ATP analysis. The number of infants who were withdrawn and eliminated from the ATP cohort at each stage of the study is presented in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A583.

Mean age of infants at the time of Dose 1 of RIX4414/placebo was 7 weeks (standard deviation = 1.06 weeks); 53% were females and the majority (99%) were Black Africans. There was no restriction on feeding; although more infants were only formula-fed (46.7% in RIX4414 group; 47.7% in placebo group) than only breast-fed (37.8% in RIX4414 group; 34.1% in placebo group) during all 3 doses of study vaccine. The baseline median absolute $CD4^+$ cell count was 2074 in the RIX4414 group and 2022 in the placebo group. The median log10 HIV-1 viral load was the same in both groups (5.9 each).

The percentage of infants who had received antiretroviral therapy at birth was 57% (31 infants in the RIX4414 group and 26 in the placebo group). None of the infants were given any other antiretroviral therapy at the beginning of study.

Safety and Reactogenicity

All symptoms (solicited and unsolicited) were recorded at a similar frequency during the 15-day follow-up period in both RIX4414 and placebo groups. In addition, fever, vomiting, or diarrhea of Grade 2 or 3 in intensity were also recorded at a similar frequency (<60%) in both groups. Cough (70% in RIX4414 and 62% in placebo) was the most commonly recorded solicited general symptom, and irritability (16% in RIX4414 and 14% in placebo) was the most commonly recorded Grade 3 solicited general symptom in both the RIX4414 and placebo groups (Fig. 1).

At least one unsolicited symptom was recorded in 94% (95% CI: 83.5–98.7) of infants in RIX4414 group and 96% (95% CI: 86.3–99.5) of infants in placebo group; at least one unsolicited symptom Graded 3 in intensity was recorded in 30% (95% CI: 17.9–44.6) of infants in each of the 2 groups during the 31-day postvaccination follow-up period. The frequently recorded unsolicited symptoms including those Graded 3 were bronchopneumonia (8% each in RIX4414 and placebo), oral candidiasis (8% in RIX4414 and 4% in placebo), and GE (8% in RIX4414 and 4% in placebo). One vaccine-related unsolicited symptom (abdominal pain) was recorded in an infant belonging to the RIX4414 group on the day the infant was administered the second dose of RIX4414 vaccine. This symptom of Grade 1 intensity lasted for 2 days.

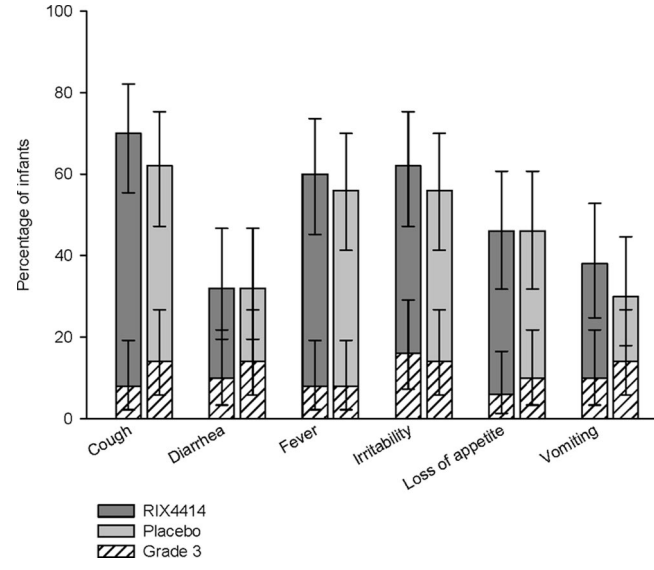

FIGURE 1. All and Grade 3 solicited general symptoms recorded during the 15-day postvaccination follow-up period (total vaccinated cohort).

TABLE 1. Immunologic Categories at Screening and 2 Months Post-Dose 3 (Total Vaccinated Cohort)

Immunologic Category	Screening				2 Months Post-Dose 3			
	RIX4414 (N = 50)		Placebo (N = 50)		RIX4414 (N = 43)		Placebo (N = 39)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Severe suppression	1	2.0 (0.1–10.6)	2	4.0 (0.5–13.7)	11	25.6 (13.5–41.2)	7	17.9 (7.5–33.5)
Moderate suppression	12	24.0 (13.1–38.2)	15	30.0 (17.9–44.6)	15	34.9 (21.0–50.9)	18	46.2 (30.1–62.8)
No suppression	37	74.0 (59.7–85.4)	33	66.0 (51.2–78.8)	13	30.2 (17.2–46.1)	10	25.6 (13.0–42.1)
Unknown	—	—	—	—	4	9.3 (2.6–22.1)	4	10.3 (2.9–24.2)

Immunologic categories: source, *Morb Mortal Wkly Rep.*[24]
N indicates number of infants; n, no. subjects in a given category; %, n/no. subjects with available results ×100.

A total of 59 SAEs were documented in 29 infants (17 in RIX4414 and 12 in placebo). Bronchopneumonia (7 in RIX4414 and 4 in placebo group) was the most commonly reported SAE during the study period and it occurred mainly after Dose 3 of RIX4414 (4/7) while in the placebo group, 2 cases each occurred after Dose 2 and Dose 3. GE (6 and 3 cases in RIX4414 and placebo, respectively) was the second most frequently reported SAE with most of the cases predominantly observed after Dose 1 of RIX4414/placebo (RIX4414 [4/6]; placebo [3/3]).

Fifteen fatal cases (6 and 9 infants in RIX4414 and placebo groups, respectively) were reported, with bronchopneumonia, sepsis, and GE being the most common cause of death. Most of the fatal SAEs were recorded post-Dose 3 of RIX4414/placebo (7/15), when compared with Dose 1(4/15) or Dose 2 (4/15) of RIX4414/placebo. None of the SAEs was considered as causally vaccine-related by the investigator.

Immunogenicity

Two months post-Dose 3, 57.1% (95% CI: 34–78.2) of infants in RIX4414 group and 18.2% (95% CI: 5.2–40.3) of infants in the placebo group had antirotavirus IgA antibody concentration ≥20 U/mL. The overall GMC in the RIX4414 group was 75.7 U/mL (95% CI: 29.1–195.7) and in the placebo group it was <20 U/mL; GMCs calculated on rotavirus seropositive infants were 344 U/mL (95% CI: 136.7–865.7) in RIX4414 group and 96.6 U/mL (95% CI: 11.1–840.1) in the placebo group. Vaccine take in RIX4414 group (15/23) was 65.2% (95% CI: 42.7–83.6), and 31.8% (95% CI: 13.9–54.9) of infants from placebo group (7/22) seroconverted and/or had vaccine virus isolated from their stool samples.

Rotavirus vaccine shedding in stool samples collected at predetermined time points demonstrated a peak at 7 days after Dose 1 of RIX4414 (37.5% in the RIX4414 group). One infant continued to shed rotavirus antigen beyond the specified follow-up period of 42 days after Dose 3 of RIX4414 vaccine. For this infant, rotavirus antigen excretion ceased between Day 56 and Day 70. In the placebo group, 5 (20%) infants shed rotavirus between Dose 1 up to study end indicating the circulation of wild-type rotavirus during the study.

GE was reported in 24 infants (48%) in the RIX4414 group and 23 infants (46%) in the placebo group. Of these, 4 infants (8%) in each group reported rotavirus GE. Analysis of the rotavirus positive GE stool samples showed 2 cases with G1P[8] wild-type, 1 case with G12P[6], and 1 case with G1P[8] (unknown strain) in the RIX4414 group. In the placebo group, 2 cases of rotavirus GE with G3P[8] and 2 cases with G2P[4] were found in the 4 stool samples.

Immunodeficiency Condition

At screening and 2 months post-Dose 3, the percentage of infants in each of the predefined immunologic categories were similar in both the study groups, indicating that vaccination with RIX4414 did not aggravate the immunodeficiency condition in the infants (Table 1).

TABLE 2. Percentage of Subjects With CD4 Cell Count and CD4 Cell Percentage Pre-Dose 1 and 2 Months Post-Dose 3 (Total Vaccinated Cohort)

	Parameters	Screening		2 Months Post-Dose 3	
		RIX4414 N = 50	Placebo N = 50	RIX4414 N = 39*	Placebo N = 35†
CD4 cell count	Median	2074	2022	1477	1427
	Minimum	547	414	106	149
	Maximum	4040	4871	4080	3716
CD4 cell percentage	Median	36.95	35.50	22.30	23.10
	Minimum	12.30	12.60	4.66	9.06
	Maximum	59.90	55.00	51.20	38.70
Log 10 (HIV-1 viral load)	Median	5.9	5.9	5.9	5.9
	Minimum	2.6	2.6	2.6	3.4
	Maximum	5.9	5.9	5.9	5.9

*No. infants in RIX4414 was 43 2 months post-Dose 3 (HIV-1 viral load).
†No. infants in Placebo group was 36 2 months post-Dose 3 (HIV-1 viral load).
HIV indicates human immunodeficiency virus.

The median absolute $CD4^+$ cell count, $CD4^+$ percentage, and log10 HIV-1 viral load were similar in both groups at screening and 2 months post-Dose 3 (Table 2). The absolute $CD4^+$ cell count and $CD4^+$ percentage decreased during the follow-up period that demonstrated the possible and expected progression of immunodeficiency condition in both the groups.

DISCUSSION

Sub-Saharan Africa continues to be the region that is considerably affected by HIV infection and it accounts for more than half of all people living with HIV throughout the world.[25] Children infected with HIV are more likely to develop opportunistic infections when compared with healthy children and it becomes essential to prevent these infections through effective vaccination. The WHO policy for immunization of HIV-infected children recommends that the DTPw, Hep B, and Hib vaccines be routinely administered at a schedule similar to that in healthy infants. However, BCG, OPV, and measles vaccine administration can be withheld for symptomatic HIV-infected infants at the discretion of the physicians.[26]

The current study was the first of its kind where the safety and immunogenicity of the live-attenuated human rotavirus vaccine, RIX4414 was evaluated in HIV-positive infants in South Africa when 3 doses of the vaccine were administered orally following the Expanded Program on Immunization (EPI) schedule

at 6, 10, and 14 weeks of age. The results of this study showed that the incidence of all symptoms (solicited and unsolicited) were similar in RIX4414 and placebo groups irrespective of the severity indicating that the RIX4414 was well tolerated, and there was no increase in reactogenicity in HIV-infected infants after vaccination with an oral live attenuated human rotavirus vaccine. None of the SAEs was vaccine-related, and the number of fatal cases did not significantly differ between the placebo group and RIX4414 group. Bronchopneumonia and GE were the common causes of both fatal and nonfatal SAEs in this study, and this is in line with other studies with this vaccine performed earlier in South Africa.[6,15]

Although the number of infants enrolled was small, these numbers were sufficient (as per sample size calculation) to validate that RIX4414 vaccine was immunogenic in this infant population. The antirotavirus IgA seroconversion rate was 57.1%, and the GMCs calculated on rotavirus seropositive infants was 344 U/mL in the RIX4414 group. These results are encouraging because the immune response is not compromised in early stage HIV-infected infants when they are immunized in the EPI schedule and in line with earlier study conducted in the same region on HIV-negative infants.[15,17] Interestingly, seroconversion and viral shedding were observed at a low level in infants who received placebo doses. It was proved by post hoc analysis that these infants had indeed acquired natural rotavirus infection during the study period as the study spanned across rotavirus seasons. In real-life conditions, rotavirus seropositivity or vaccine take will always be a composite of both the immune response directly linked to vaccination and a boosting effect related to exposure to circulating wild-type rotavirus. Even though this study is not able to reveal the exact fraction of the immune response directly linked to the vaccine, the difference in point estimates of antirotavirus IgA GMCs observed 2 months after vaccination between vaccinees (344 U/mL) and placebo recipients (96.6 U/mL) supports the substantial priming effect of the vaccine doses. This evidence has been confirmed in the other studies conducted in the same population.[15,16]

Peak rotavirus vaccine shedding was seen on Day 7 after Dose 1 of RIX4414 vaccine, which is similar to that reported in a study conducted in healthy infants.[27] The duration of rotavirus antigen excretion in HIV-positive infants appeared to be similar to that observed in healthy infants with prolonged shedding observed in only 1 infant in the RIX4414 vaccine group (rotavirus shedding beyond Day 42 post-Dose 3). This was of particular concern, given earlier observations of an increase in shedding of wild-type rotavirus in HIV-infected infants in Malawi.[8]

The HIV status was also assessed to evaluate if the rotavirus vaccine had any effect on the immunosuppressive progression. Assessment of immunodeficiency condition in terms of absolute $CD4^+$ cell count, $CD4^+$ percentage, and log 10HIV-1 viral load at screening and after vaccination showed that there was no difference observed in these parameters between the study groups. This indicates that the RIX4414 vaccine does not aggravate the immunodeficiency condition in HIV-positive infants. This was also observed in the natural wild-type rotavirus infection in HIV-infected infants.[8,9] As expected, it was noticed that during the course of the study there was a gradual decrease in the $CD4^+$ cell count and in $CD4^+$ percentage in both groups depicting the progression of the HIV infection in this infant population.

In line with the WHO recommendation, rotavirus vaccination has been included as part of the routine EPI worldwide, thus paving the way for universal mass vaccination in the African region as well.[7] However, for the potentially immunocompromised and symptomatic HIV-infected infants, WHO recommends that rotavirus vaccine can be administered at the discretion of the physicians.[28] Although this strategy may apply to the private sector, it is unlikely to be manageable in the public health sector in sub-Saharan Africa. Considering the high HIV carrier-rate in women attending antenatal clinics in this population, introduction of mass rotavirus vaccination of all infants is expected to help in reducing the overall rotavirus disease burden in this region, and potentially contributing to alleviating unnecessary suffering in this vulnerable population.

This study supports rotavirus vaccination in HIV-positive infants as it demonstrates good immunogenicity with no safety issues and without any apparent effect on the immunodeficiency condition of the vaccinees.

ACKNOWLEDGMENTS

The authors thank the infants and their families for participating in this trial, all the investigators, clinicians, study nurses, and other staff members for contributing in many ways to these studies. The authors thank Leen-Jan van Doorn and his team from DDL Diagnostic Laboratory (The Netherlands) for performing reverse transcriptase polymerase chain reaction (RT-PCR) followed by reverse hybridization to determine rotavirus G and P types, Dr. Richard Ward and Nicole Meyer at the Laboratory for Specialized Clinical Studies, Cincinnati Children's Hospital Medical Center (Cincinnati, OH) for rotavirus ELISA antigen testing and the Medical Research Council unit at University of Limpopo (Medunsa), Pretoria for performing RT-PCR followed by gel detection to determine G and P types, Catherine Bougelet and team for laboratory testing, Isabelle Stainier and Jean-Marc Delroisse for the molecular analysis (full-genome sequencing) performed on the stool samples (when appropriate), Yolanda Guerra and safety team for management of safety information, Aurore Dujardin and Saartje Vansteenkiste for global study management, Vincent Melot and Lynette Wane for country study management, Silvia Damaso for providing statistical input, Geetha Subramanyam for assistance in writing the manuscript, Nancy Van Driessche and Manjula K for editorial assistance and manuscript coordination (all from GlaxoSmithKline Biologicals).

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